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Review

Crimean–Congo hemorrhagic fever

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Abstract

Crimean–Congo hemorrhagic fever (CCHF) is a tick-borne disease caused by the arbovirus Crimean–Congo hemorrhagic fever virus (CCHFV), which is a member of the *Nairovirus* genus (family *Bunyaviridae*). CCHF was first recognized during a large outbreak among agricultural workers in the mid-1940s in the Crimean peninsula. The disease now occurs sporadically throughout much of Africa, Asia, and Europe and results in an approximately 30% fatality rate. After a short incubation period, CCHF is characterized by a sudden onset of high fever, chills, severe headache, dizziness, back, and abdominal pains. Additional symptoms can include nausea, vomiting, diarrhea, neuropsychiatric, and cardiovascular changes. In severe cases, hemorrhagic manifestations, ranging from petechiae to large areas of ecchymosis, develop. Numerous genera of ixodid ticks serve both as vector and reservoir for CCHFV; however, ticks in the genus *Hyalomma* are particularly important to the ecology of this virus. In fact, occurrence of CCHF closely approximates the known world distribution of *Hyalomma* spp. ticks. Therefore, exposure to these ticks represents a major risk factor for contracting disease; however, other important risk factors are known and are discussed in this review. In recent years, major advances in the molecular detection of CCHFV, particularly the use of real-time reverse transcription-polymerase chain reaction (RT-PCR), in clinical and tick samples have allowed for both rapid diagnosis of disease and molecular epidemiology studies. Treatment options for CCHF are limited. Immunotherapy and ribavirin have been tried with varying degrees of success during sporadic outbreaks of disease, but no case-controlled trials have been conducted. Consequently, there is currently no antiviral treatment for CCHF approved by the U.S. Food and Drug Administration (FDA). However, renewed interested in CCHFV, as well as increased knowledge of its basic biology, may lead to improved therapies in the future. This article reviews the history, epidemiology, ecology, clinical features, pathogenesis, diagnosis, and treatment of CCHF. In addition, recent advances in the molecular biology of CCHFV are presented, and issues related to its possible use as a bioterrorism agent are discussed.

Keywords: Crimean–Congo hemorrhagic fever; Tick-borne virus; Epidemiology, pathogenesis and treatment of CCHF

Contents

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14. ABSTRACT

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne disease caused by the arbovirus Crimean-Congo hemorrhagic fever virus (CCHFV), which is a member of the Nairovirus genus (family Bunyaviridae). CCHF was first recognized during a large outbreak among agricultural workers in the mid-1940s in the Crimean peninsula. The disease now occurs sporadically throughout much of Africa, Asia, and Europe and results in an approximately 30% fatality rate. After a short incubation period, CCHF is characterized by a sudden onset of high fever, chills, severe headache, dizziness, back, and abdominal pains. Additional symptoms can include nausea, vomiting, diarrhea, neuropsychiatric, and cardiovascular changes. In severe cases, hemorrhagic manifestations, ranging from petechiae to large areas of ecchymosis, develop. Numerous genera of ixodid ticks serve both as vector and reservoir for CCHFV; however, ticks in the genus Hyalomma are particularly important to the ecology of this virus. In fact, occurrence of CCHF closely approximates the known world distribution of Hyalomma spp. ticks. Therefore, exposure to these ticks represents a major risk factor for contracting disease; however, other important risk factors are known and are discussed in this review. In recent years, major advances in the molecular detection of CCHFV, particularly the use of real-time reverse transcription-polymerase chain reaction (RT-PCR), in clinical and tick samples have allowed for both rapid diagnosis of disease and molecular epidemiology studies. Treatment options for CCHF are limited. Immunotherapy and ribavirin have been tried with varying degrees of success during sporadic outbreaks of disease, but no case-controlled trials have been conducted. Consequently, there is currently no antiviral treatment for CCHF approved by the U.S. Food and Drug Administration (FDA). However, renewed interested in CCHFV, as well as increased knowledge of its basic biology, may lead to improved therapies in the future. This article reviews the history, epidemiology, ecology, clinical features, pathogenesis, diagnosis, and treatment of CCHF. In addition, recent advances in the molecular biology of CCHFV are presented, and issues related to its possible use as a bioterrorism agent are discussed.

15. SUBJECT TERMS

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1. Historical perspective

1.1. Early history of Crimean–Congo hemorrhagic fever (CCHF)

A disease now considered to be CCHF was described by a physician in the 12th century from the region that is presently Tadzhikistan. The description was of a hemorrhagic disease with the presence of blood in the urine, rectum, gums, vomitus, sputum, and abdominal cavity and was said to be caused by a louse or tick, which normally parasitizes a blackbird (Hoogstraal, 1979). The arthropod described may well have been a species of *Hyalomma* tick larvae which are frequently found on blackbirds. CCHF has also been recognized for centuries under at least three names by the indigenous people of southern Uzbekistan: *khungribta* (blood taking), *khunymuny* (nose bleeding), or *karakhalak* (black death) (Chumakov, 1974; Hoogstraal, 1979). [The term "black death," now commonly used to refer to plague (*Yersina pestis*), did not appear in the Oriental literature on plague, and was not commonly used in European languages until the 16th and 17th centuries (Dols, 1977).] In Central Asia, various hemorrhagic diseases including acute infectious capillarotoxicosis, acute infectious hemorrhagic disease, and Uzbekistan hemorrhagic fever have been known for centuries to produce a disease similar to CCHF (Chumakov et al., 1976).

1.2. Discovery of the virus

Crimean hemorrhagic fever (CHF) came to the attention of modern medical science and was first described as a clinical entity in 1944–1945 when about 200 Soviet military personnel were infected during an epidemic in war-torn Crimea (Chumakov, 1945, 1947). Shortly thereafter, a viral etiology was suggested by reproducing a febrile syndrome in psychiatric patients undergoing pyrogenic therapy after inoculation with a filterable agent from the blood of CHF patients (Chumakov, 1974). Further evidence of a viral etiology and of a suspected tick-borne route of infection was demonstrated by inducing a mild, but characteristic, clinical course of CHF in healthy human volunteers 2 days after their inoculation with filtered suspensions of nymphal *Hyalomma marginatum* ticks in the presence of antibiotics (Chumakov, 1974).

In 1967, a breakthrough in CHF research came when Chumakov and his colleagues at the Institute of Poliomyelitis and Viral Encephalitides in Moscow first used newborn white mice for CHF virus isolation (Butenko et al., 1968; Chumakov et al., 1968). The resulting Drosdov strain, isolated by this method from a patient (Drosdov) in Astrakhan, became the prototype strain for much experimental work in Russia and elsewhere. This now gave researchers an actual virus to use in a variety of experimental studies and allowed for the production of the necessary reagents (i.e., antibodies and antigens) needed for serological surveys and for the identification and classification of viral isolates obtained from various geographic regions. In fact, several agents of tick-borne hemorrhagic fevers from Kazakhstan and Uzbekistan and from various areas across Africa were found to be indistinguishable from each other. This ultimately led to work showing CHF virus was antigenically indistinguishable from the Congo virus (Casals, 1969; Chumakov et al., 1969), a virus originally isolated from human patients from the Congo and Uganda (Simpson et al., 1967; Woodall et al., 1967). The realization that the Congo virus and CHF virus were,

in fact, the same virus lead to the new name, CHF–Congo virus. However, many authors found the name awkward, and have adopted Crimean–Congo hemorrhagic fever virus (Hoogstraal, 1979).

2. Classification of the virus

CCHFV is a member of the *Nairovirus* genus of the family *Bunyaviridae*. Other genera within the family include *Orthobunyavirus*, *Hantavirus*, *Phlebovirus*, and *Tospovirus*. According to the most recent report from the International Committee on the Taxonomy of Viruses, there are seven recognized species in the genus *Nairovirus* containing 34 viral strains (Elliott et al., 2000), all of which are believed to be transmitted by either ixodid or argasid ticks (i.e., hard or soft ticks, respectively). The most important serogroups are the CCHF group, which includes CCHFV, and Hazara virus, which has not been demonstrated to be pathogenic to humans, and the Nairobi sheep disease group, which includes Nairobi sheep disease (NSD) and Dugbe viruses. Only three members are known to be pathogens of humans, namely, CCHFV, Dugbe and Nairobi sheep disease viruses, although the latter is primarily a pathogen of sheep and goats. Dugbe virus causes a mild febrile illness and thrombocytopenia in humans (Burt et al., 1996).

3. Structure and molecular biology of the virus

Relatively few studies have been made on the structure of CCHFV. Murphy et al. (1968, 1973) first described the morphology of CCHFV in the brains of infected newborn mice and noted the similarity to members of the *Bunyaviridae* family. Indeed, it is now known that CCHFV, and nairoviruses in general, are typical of other members of the family *Bunyaviridae* in terms of their basic structure, morphogenesis, replication cycle, and physicochemical properties (Donets et al., 1977; Ellis et al., 1981; Martin et al., 1985; Swanepoel, 1995; Schmaljohn and Hooper, 2001). The principal stages of the replication process for viruses in the *Bunyaviridae* are similar to those of many other enveloped viruses and are illustrated in Fig. 1. Virions are spherical, approximately 100 nm in diameter, and have a host cell-derived lipid bilayered envelope approximately 5–7 nm thick, through which protrude glycoprotein spikes 8–10 nm in length (Fig. 2) (Marriott and Nuttall, 1996a; Swanepoel, 1995; Schmaljohn and Hooper, 2001). When viewed by negative stain electron microscopy, CCHF virions appear to be distinct from other viruses within the *Bunyaviridae* family, as they possess very small morphologic surface units with no central holes arranged in no obvious order (Martin et al., 1985). Virions of members of the family *Bunyaviridae* contain three structural proteins: two envelope glycoproteins (G2 and G1 [more recently termed

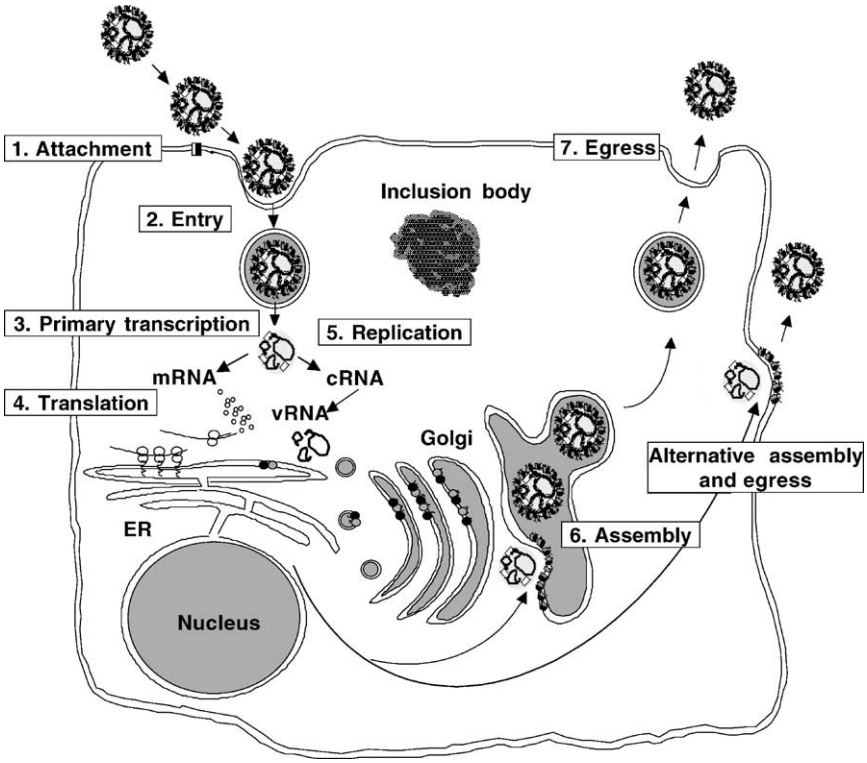

Fig. 1. Replication cycle of viruses in the family *Bunyaviridae*. Steps in the replication cycle are numbered as follows: (1) attachment of virions to cell-surface receptors; (2) entry via endocytosis followed by membrane fusion, allowing viral ribonucleocapsids and RNA-dependent RNA polymerase access to the cytoplasm; (3) primary transcription; (4) translation of viral proteins; (5) replication of vRNA via a cRNA intermediate; (6) assembly of virions at the Golgi or plasma membrane; (7) egress by budding into the Golgi followed by exocytosis, or budding through the plasma membrane. ER, endoplasmic reticulum. Reproduced from Schmaljohn and Hooper (2001) with permission.

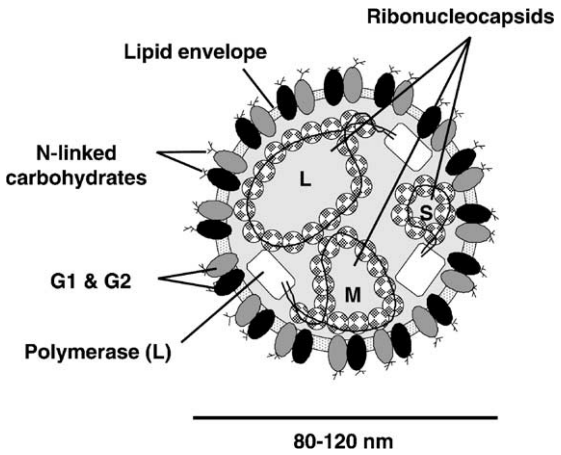

Fig. 2. Schematic cross-section of a *Bunyaviridae* virion. The three RNA genome segments (S, M, and L) are complexed with nucleocapsid protein to form ribonucleocapsid structures. The nucleocapsids and RNA-dependent RNA polymerase are packaged within a lipid envelope that contains the viral glycoproteins, G1 and G2 (also referred to as Gn and Gc, respectively). Reproduced from Schmaljohn and Hooper (2001) with permission.

Gn and Gc, respectively, named in accordance with their relative proximity to the amino or carboxy terminus of the M segment encoded polyprotein]) and a nucleocapsid protein (N), plus a large polypeptide (L) (approximately 200 kDa), which is the viron-associated RNA-dependent RNA polymerase (Schmaljohn and Hooper, 2001; Marriott and Nuttall, 1996b). Recently, two independent research groups published the complete nucleotide sequence of the CCHFV L segment (Honig et al., 2004; Kinsella et al., 2004). The sequence is approximately 60% identical both at the nucleotide and amino acid levels to the L segment of Dugbe virus, the only other *Nairovirus* genome to be fully sequenced, with the most highly conserved area being that encoding the region corresponding to the core catalytic domains of the RNA-dependent RNA polymerase. Further analysis of the L segment sequences from both CCHFV and Dugbe virus revealed the presence of a zinc-finger domain and a leucine zipper motif, suggesting that nairovirus L segments display characteristics of viral helicases (i.e., having both helicase activity and polymerase activity stemming from one polyprotein), most often seen in positive-strand RNA virus replicases. Furthermore, high-sequence homology with a newly formed superfamily of predicted cysteine proteases, termed ovarian tumor (OTU)-like proteases, was discovered, which was also suggested from the L segment sequence of Dugbe virus (Makarova et al., 2000). From these data, it is hypothesized that the OTU-like protease may function in the *Nairovirus* L protein by autoproteolytically cleaving the polyprotein to yield a polymerase and a helicase (Honig et al., 2004; Kinsella et al., 2004). Other suggested functions of the OTU-like protease include involvement with deubiquitination activitites, such that has recently been demonstrated for the adenovirus L3 23 K proteinase (Balakirev et al., 2002). Clearly, this is only the beginning of future studies to elucidate the complex nature of the protein products encoded by the CCHFV L segment.

The genome is characteristic of other members of the family and is composed of three negative-strand RNA segments, S, M, and L, encoding the N nucleocapsid, Gn and Gc glycoproteins, and the L polymerase, respectively. The RNA segments are complexed with N to form individual S, M, and L nucleocapsids, which appear to be circular or loosely helical (Bishop, 1996). The M segment of nairoviruses is 30–50% larger than the M segments of members of other genera in the *Bunyaviridae* family and has the potential coding capacity of up to 240 kDa of protein (Elliott, 1990). At least one of each of the S, M, and L ribonucleocapsids must be contained in a virion for infectivity; however, equal numbers of nucleocapsids may not always be packaged in mature virions (Schmaljohn and Hooper, 2001). Recent data show that the N protein is targeted to the perinuclear region of infected cells in the absence of native RNA segments and that this targeting is actin filament dependent (Andersson et al., 2004a,b). The first 8–13 nucleotide bases at the 3′ termini of all three RNA segments have a sequence (3′-AGAGUUUCU. . .) that is conserved within viruses of the genus (Clerex-van Haaster et al., 1982), with a complementary consensus sequence at the 5′ termini. Base-pairing of the terminal nucleotides is predicted to form stable panhandle structures and noncovalently closed circular RNAs, which have been directly observed by electron microscopy of RNA extracted from another bunyavirus, Uukuniemi, virions (Hewlett et al., 1977).

The viral glycoproteins are believed to be responsible for recognition of receptor sites on susceptible cells. Viruses which attach to receptors on susceptible cells are internalized by endocytosis, and replication occurs in the cytoplasm (see Fig. 1). Virions mature by budding through endoplasmic reticulum into cytoplasmic vesicles in the Golgi region, which are presumed to fuse with the plasma membrane to release virus (Donets et al., 1977; Ellis et al., 1981). Much recent work has been done on the molecular characterization of the glycoproteins of CCHFV. Sanchez et al. (2002) demonstrated that during CCHFV infection, the mature Gn (37-kDa) and Gc (75-kDa) proteins form the predominant structural glycoprotein components of the virus. Additionally they showed that the M RNA segment of CCHFV encodes a polyprotein, which undergoes proteolytic processing to yield a 140-kDa precursor protein of Gn (PreGn, previously referred to PreG2) and an 85-kDa precursor protein of Gc (PreGc, previously referred to as PreG1). It was recently shown that CCHFV uses, at least in part, the subtilase SKI-1 and possibly related cellular proteases for the major glycoprotein precursor cleavage events (Sanchez et al., 2002; Vincent et al., 2003), as has been demonstrated for the *Arenavirus*, Lassa virus (Lenz et al., 2001).

Recently, a reverse genetics system was developed for CCHFV (Flick et al., 2003), which was based on the RNA polymerase I transcription system recently used in the development of a reverse genetics system for Uukuniemi virus (Flick and Pettersson, 2001). The development of a reverse

genetics system for CCHFV was a major step forward in efforts to understand the biology of the virus. The development of an infectious clone for CCHFV will allow for more extensive studies of its biology and pathogenesis, and may ultimately lead to better therapeutic and prophylactic measures against CCHFV infections.

4. Strain variation and phylogenetic relationships

Many early studies, based on serological testing, suggested that there are very few significant differences among strains of CCHFV. For example, studies employing modified agar gel diffusion precipitation, neutralization, cell culture interference, and complement fixation tests demonstrated that there were no apparent antigenic differences among strains from several different geographic locations in the former Soviet Union and Africa (Casals, 1969; Casals et al., 1970; Chumakov et al., 1969; Tignor et al., 1980). However, more recent data based on nucleic acid sequence analysis have revealed extensive genetic diversity. The first published CCHFV sequence data was of the S RNA segment (which encodes the viral nucleoprotein) of the Chinese sheep isolate C68031 (Marriott and Nuttall, 1992). Since then, several additional S segment sequences from CCHFV isolates from different regions of the world have been published (Schwarz et al., 1996; Rodriguez et al., 1997; Papa et al., 2002a; Drosten et al., 2002b; Yashina et al., 2003). Analysis of these sequences show considerable genetic differences (Fig. 3). For example, several CCHFV S segment sequences of PCR products obtained directly from infected patients from the 1994–1995 outbreak in the United Arab Emirates (UAE) showed a divergence of 10.0–11.8% when compared with the Chinese sheep isolate C68031; however, most of the nucleotide changes were in the third position (Schwarz et al., 1996). Two isolates obtained from Kosovo in 2001 (Papa et al., 2002b) showed a 17% difference in nucleotide sequence in the S segment from the Nigerian strain IbAr 10200, while differing only by 4% from the Drosdov strain, originally isolated from the blood of a patient in Russia. Likewise, strains obtained in neighboring Albania were closely related to those from Kosovo and phylogenetically clustered together along with the Drosdov strain from Russia (Papa et al., 2002a). It is interesting that the Greek strain AP92 differed greatly from other European strains (e.g., 24.3% and 25.3% nucleotide difference from the Kosovo and Albanian strains, respectively), and therefore, clusters in a group by itself (Fig. 3). AP92 strain was originally isolated in Greece from a *Rhipicephalus bursa* tick and has not yet been associated with disease in humans. Also, strains from China are known to be greatly divergent from African strains. Two Chinese strains (BA66019 and BA8402) exhibited a 15% difference in nucleotide sequence in the S segment and a mean difference of 22% in the M segment from those of the Nigerian strain IbAr 10200 (Papa et al., 2002c). Additionally, a strain from southern Russia (STV/HU29223) and one from Uzbekistan (TI10145) differed in their S segment sequence by 14.9% and 13.2%, respectively, from the Nigerian IbAr 10200. Based on the S RNA sequence, the southern Russian strain was most closely related to Drosdov (4.7% difference) and the sequence of the Uzbekistan strain was most closely related to the Chinese strains (3.8% and 3.7% difference from strain 8402 and HY13, respectively) (Yashina et al., 2003).

More recent work has begun to shed light on the genetic diversity of the M RNA segment. The first published characterization of the CCHFV M RNA segment was of the Chinese strains, BA66019 and BA8402, isolated in 1965 and 1984 from a CCHF patient and *Hyalomma* spp. ticks, respectively (Papa et al., 2002c), although a complete M RNA sequence of the reference strain IbAr 10200 was deposited in GenBank (accession number U39455) previously. The coding nucleotide sequences of the two Chinese strains differed for the Nigerian strain IbAr 10200 by a mean of 22%, supplying further evidence of the extent of genetic diversity among these viruses. Recently, sequence analysis of the M RNA segments from CCHFV isolates from Russia and from Central Asia (Tajikistan) indicated that they each form separate phylogenetically distinct groups (Yashina et al., 2003; Seregin et al., 2004).

5. Ecology and epidemiology of CCHFV

5.1. Vertebrate reservoir hosts

Like other tick-borne zoonotic agents, CCHFV generally circulates in nature unnoticed in an enzootic tick–vertebrate–tick cycle. CCHFV has been isolated from numerous domestic and wild vertebrates, including cattle and goats (Woodall et al., 1965; Causey et al., 1970), sheep (Yu-Chen et al., 1985), hares (Chumakov, 1974), hedgehogs (Causey et al., 1970), a *Mastomys* spp. mouse (Saluzzo et al., 1985), and even domestic dogs (Shepherd et al., 1987a,b). Sera from several species of wild mammals have antibodies to CCHFV and seroepidemiological studies have also detected antibodies to CCHFV in domestic cattle, horses, donkeys, sheep, goats, and pigs from various parts of Europe, Asia, and Africa (Watts et al., 1989a,b). Interestingly, there has been only one report of antibody to CCHFV detected from a reptile, a tortoise from Tadzhikistan (Pak et al., 1971) even though immature *Hyalomma anatolicum* ticks, a common CCHFV vector, are known to sometimes feed on lizards (Hoogstraal, 1979). For a comprehensive listing of vertebrates from which either CCHFV has been isolated or antibody to CCHFV detected, the reader is referred to Hoogstraal (1979) and Watts et al. (1989a,b).

Although many domestic and wild vertebrates are infected with CCHFV, as evidenced by development of viremia and/or antibody response, birds, in general, appear to be refractory to infection with CCHFV. For instance, early experiments by Berezin et al. (1971a,b) showed that after experimental inoculation of birds (rooks and rock doves) with CCHFV,

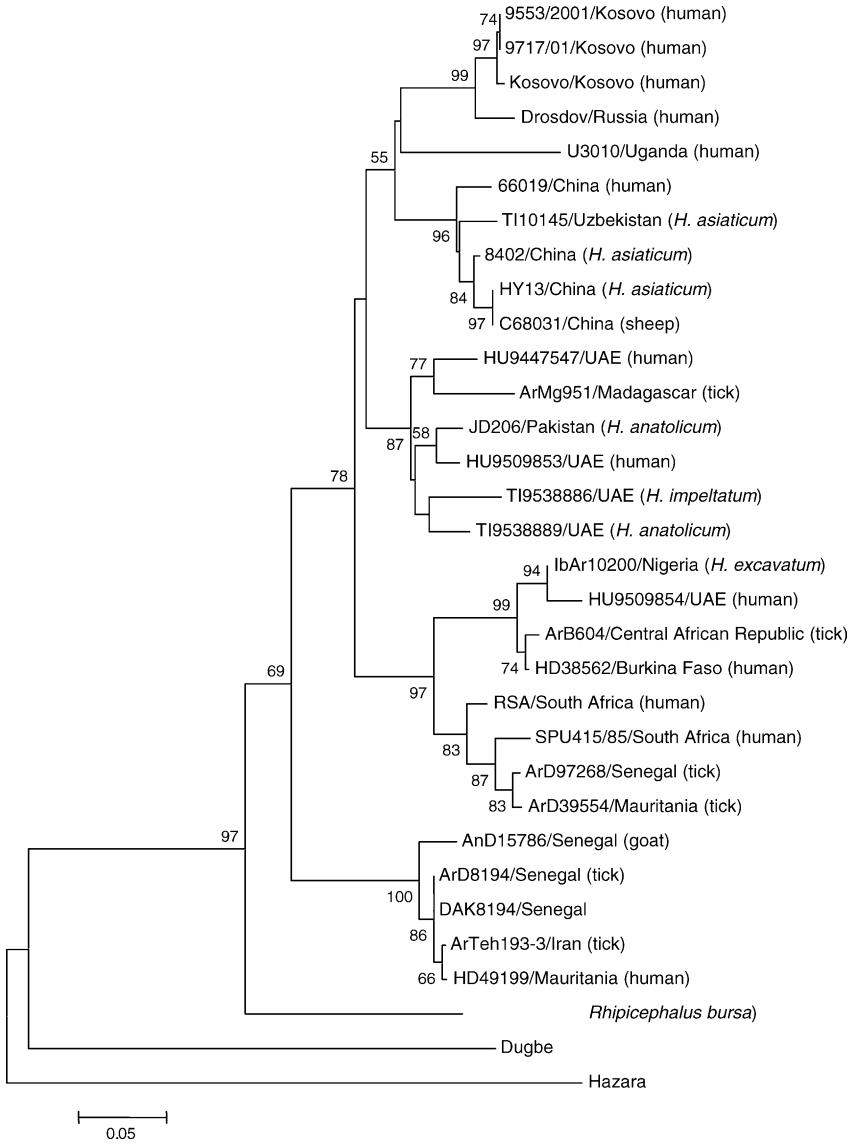

Fig. 3. Phylogenetic relationships inferred from comparing partial sequences of the S segment RNA of CCHFV. Sequences were aligned by the multisequence alignment program GeneDoc (version 2.6.002) and analyzed by a neighbor-joining method with Kimura two-parameter distances by using MEGA software (version 2.1). The lengths of the horizontal branches are proportional to the number of nucleotide differences between taxa. Vertical branches are for visual clarity only. Bootstrap values above 50%, obtained from 500 replicates of the analysis, are shown at the appropriate branch points. CCHFV strains are described as strain designation/country of origin. The GenBank accession numbers of the CCHFV S RNA segment sequences used are as follows: IbAr 10200 (U88410); Drosdov, HY13, and JD206 (U88412, U88413 and U88414, respectively); 9553/2001 (AF428144), 9717/01 (AF428145), Kosovo (AF404507), U3010 (U88416), 66019 (AJ010648), TI10145 (AF481799), 8402 (AJ010649), C68031 (M86625), HU9447547 (U75670), ArMg951 (U15024), HU9509853 (U75672), TI9538886 (U75673), TI9538889 (U75669), HU9509854 (U75671), ArB604 (U15092), HD38562 (U15093), RSA (U75675), SPU415/85 (U88415), ArD97268 (U15091), ArD39554 (U15089), AnD15786 (U15020), ArD8194 (U1502), DAK8194 (U88411), ArTeh193-3 (U15022), HD49199 (U15023), AP92 (U04958). Two outgroup taxa included Dugbe virus (strain KT 281/75, AF434165) and Hazara virus (strain JC280, M86624). Scale bar, 5% divergence.

they remained healthy, and evidence of viremia or an antibody response could not be demonstrated. Furthermore, work by the same group, showed that even though CCHFV could be isolated from nymphal ticks collected from over 600 birds, the birds remained serologically negative for antibody to CCHFV. Attempts to isolate virus from the blood and organs of 360 of those birds were uniformly negative. Several additional examples are known from the 1970s in which CCHFV has been isolated from ticks infecting numerous species of birds, which remain serologically negative for the virus (Hoogstraal, 1979). Taken together, these studies suggest that birds appear to be refractory to CCHF viremia even though they can support large numbers of CCHFV-infected ticks. However, some exceptions do exist; detected antibodies to CCHFV in 1 of 428 sera tested from chickens and ducks in Kazakhstan and also detected CCHFV antibodies in the serum of a magpie (*Pica pica*). However, in more recent pathogenicity studies, domestic chickens proved to be refractory to CCHFV infection (Shepherd et al., 1987a,b). Also, in the same study, low CCHF viremia was detected

in a blue-helmeted guinea fowl (*Numidia meleagris*) after experimental infection with CCHFV. In another more recent study, antibodies were detected after CCHFV inoculation in a red-beaked hornbill and a glossy starling (but not in two laughing doves or six domestic chickens); however, none of the birds showed detectable viremia (Zeller et al., 1994).

Another interesting exception has been the disease's apparent association with the commercial ostrich meat industry in South Africa. In 1984, a case of CCHF occurred in a worker who became ill after slaughtering ostriches (*Struthio camelus*) on a farm in South Africa (Van Eeden et al., 1985). Antibody to CCHFV was detected in 24% of ostriches from surrounding farms, including six of nine ostriches from the farm where the patient worked. Interestingly, none of 460 birds of 37 other species tested during that study had detectable antibodies to CCHFV (Shepherd et al., 1987a,b). Also, in 1996, there was an outbreak of 17 cases of CCHF among workers at an ostrich abattoir (Swanepoel et al., 1998). In both instances, it was suspected that infection was acquired either by contact with ostrich blood or by inadvertently crushing infected ticks while skinning the ostriches. Ostriches have also been experimentally infected with CCHFV (Swanepoel et al., 1998). The ostriches, which were experimentally infected with CCHFV subcutaneously, developed viremia 1–4 days after infection and virus was detectable in visceral organs up to 5 days post-inoculation. It was concluded from these studies that infection in ostriches at abattoirs could be prevented by keeping the birds free of ticks for a certain period of time before slaughter. This led to the standard 30-day pre-slaughter quarantine period currently enforced in South African ostrich export facilities.

Clearly, ground-feeding birds may play an important role in the ecology and epizootiology of CCHF by transporting virus-infected ticks (even though the birds themselves may remain non-viremic). However, the role, if any, for the birds themselves are not clear and additional work needs to be done in this area to resolve these issues.

In summary, vertebrates are essential as a source of blood for vector ticks and the number of species of vertebrates implicated in the natural history of CCHF is extensive, the exact role, if any, of vertebrates in the maintenance and transmission of the virus remains to be determined.

5.2. Tick vectors

CCHFV has been isolated from at least 31 species of ticks and one species of biting midge (*Culicoides* spp.) (Hoogstraal, 1979; Linthicum and Bailey, 1994). Viral isolations from ticks have been made from two species in the family Argasidae (soft ticks) and from seven genera of the family Ixodidae (hard ticks). Viral isolation alone from a tick species, however, does not incriminate them as vectors. In many cases, in fact, there is no definitive evidence that these arthropods are capable of serving as vectors for the virus, but simply virus may be present in a recent blood meal from a viremic host. For example, the one instance where virus was isolated from a biting midge, the midge was collected by a light trap near a cattle shed in Nigeria and may have contained undigested blood (Causey et al., 1970). Similarly, it seems unlikely that argasids are capable of transmitting CCHFV since the virus failed to replicate in three species of soft ticks (i.e., *Argas walkerae*, *Ornithodoros savignyi*, and *Ornithodoros porcinus*) after intracoelomic inoculation (Shepherd et al., 1989) and the same was shown for the soft tick *Ornithodoros sonrai* (Durden et al., 1993). Far more important to the ecology and epidemiology of CCHF are ticks in the genus *Hyalomma*. As early as 1944, *Hyalomma* spp. ticks were implicated in the ecology of CCHF based upon a relationship between clinical cases and tick bite. In fact, the following year, a healthy volunteer subcutaneously inoculated with a suspension of 370 nymphal *H. marginatum* ticks developed a disease characteristic of mild CCHF. This not only helped to prove the viral etiology of this disease, but also implicated *Hyalomma* spp. ticks as possible vectors; however, it was not until the late 1960s that the virus was isolated from adult *Hyalomma*, as well as several other tick species (Hoogstraal, 1979; Chumakov, 1971). In general, the known occurrence of CCHFV in Europe, Asia, and Africa coincides with the world distribution of ticks of the genus *Hyalomma* (Hoogstraal, 1956, 1979; Watts et al., 1989a,b). CCHF viral replication and tissue tropism in *Hyalomma truncatum* ticks were examined by Dickson and Turell (1992). In ticks that were experimentally infected with CCHFV, virus was recovered in highest titers from salivary glands and reproductive tissues and was positively associated with blood feeding. Additionally, virus was recovered from Malpighian tubules, midgut, muscle, and nervous tissues from nearly all the ticks tested; however, viral titers were consistently low from these tissues. Although *Hyalomma* spp. ticks are considered the most important in the epidemiology of CCHF, the virus has been isolated from ticks in other genera (i.e., *Rhipicephalus*, *Boophilus*, *Dermacentor*, and *Ixodes* spp.) as well, which may contribute to its wide geographical distribution. The biological role of ticks is also important, not only as virus vectors, but also as reservoirs of the virus in nature. Evidence of this phenomenon for CCHFV is based mainly on limited viral isolations from the eggs of field-collected *Hyalomma* spp. and *Dermacentor* spp. ticks and isolation of CCHFV from unfed ticks in the spring (Chumakov, 1965, 1972; Pak et al., 1974). Because these ticks were unfed, they must have acquired the virus from their infected mother, passed through the eggs (transovarial transmission). In addition, virus can be passed directly from immature ticks to subsequent life stages (transstadial transmission) (i.e., from larvae to nymph to adult), and this has been shown experimentally with CCHFV for several species of ticks (Logan et al., 1989; Okorie, 1991; Shepherd et al., 1991; Gordon et al., 1993; Dohm et al., 1996). Interestingly, venereal transmission of CCHFV from male to female *H. truncatum* has also been observed (Gonzalez et al., 1992).

Fig. 4. The worldwide geographic distribution of CCHF viral isolates and human disease.

5.3. Geographical distribution

The known distribution of CCHFV covers the greatest geographic range of any tick-borne virus and there are reports of viral isolation and/or disease from more than 30 countries in Africa, Asia, southeast Europe, and the Middle East (Fig. 4) (Hoogstraal, 1979; Swanepoel, 1995). Evidence for its presence in France, Portugal, Egypt, and India is based only on limited serologic observations. Interestingly, after several decades of only serologic evidence of the existence CCHFV in Turkey, an outbreak of disease in the eastern Black Sea region of the country was recently reported (Karti et al., 2004). Additionally, viral isolates were made from two of the patients, and phylogenetic analysis of the isolates suggests that two different genetic lineages of CCHFV are circulating in Turkey. These closely resemble virus lineages found in Kosovo and southwestern Russia and are clearly distinct from those associated with a recent CCHF outbreak in neighboring Iran in 2002 (Mardani et al., 2003), consistent with CCHFs being enzootic in Turkey, rather than having been introduced from Iran by infected tick or livestock movement.

6. Clinical features

Humans appear to be the only host of CCHFV in which disease is manifested (except for newborn mice). In contrast to the inapparent infection in most other vertebrate hosts, human infection with CCHFV often results in severe hemorrhagic disease. The historical accounts of disease attributed to CCHF have been reviewed in detail by Hoogstraal (1979). The typical course of CCHF has been noted by some authors as progressing through four distinct phases, i.e., *incubation*, *prehemorrhagic*, *hemorrhagic*, and *convalescence* (Hoogstraal, 1979); however, it is noteworthy that the duration and associated symptoms of these phases can vary greatly. In general, the incubation period after a tick bite can be as short as 1–3 days, but can much longer, depending on several factors including route of exposure. For example, in South Africa, among 21 patients for which reliable data were obtained, the time to onset of disease after exposure by tick bite was 3.2 days, to blood or tissue of livestock was 5.0 days, and to blood of human cases was 5.6 days (Swanepoel et al., 1987). It has been hypothesized that different hosts can induce phenotypic changes in CCHFV strains that modulate viral virulence (Gonzalez et al., 1995). It is unclear whether the variation observed in incubation times, and ultimately disease outcome, may be due to this phenomenon or other factors, such as viral dose. After the incubation period, the prehemorrhagic period is characterized by a sudden onset of fever, chills, severe headache, dizziness, photophobia, and back and abdominal pains. Additional symptoms such as nausea, vomiting, diarrhea and an accompanying loss of appetite are common. Fever is often very high (39–41 °C) and can be constantly elevated for 5–12 days or may be biphasic. It is interesting that neuropsychiatric changes have been reported in some CCHF patients. These have included sharp changes in mood, with feelings of confusion and aggression and even some bouts of violent behavior (Swanepoel et al., 1987, 1989). Cardiovascular changes can also be seen and include bradycardia and low blood pressure. This is not always the case, however; of the 11 cases of CCHF during an outbreak in the UAE from 1994 to 1995, none had signs of cardiovascular abnormalities, although eight (72.7%) ultimately died (Schwarz et al., 1997).

In severe cases, 3–6 days after onset of disease, hemorrhagic manifestations develop. These can range from petechiae to large areas of ecchymosis and often appear on the mucous membranes and skin, especially on the upper body and/or extremities (Fig. 5). Bleeding in the form of melena, hematemesis, and epistaxis is also commonly seen by day 4 or 5 and can often be characterized by dark “coffee grounds” vomitus and tar-like stools resulting from intestinal hemorrhages. Bleeding from other sites including the vagina, gingival bleeding and, in the most severe cases, cerebral hemorrhage have been reported (Swanepoel et al., 1987). Not

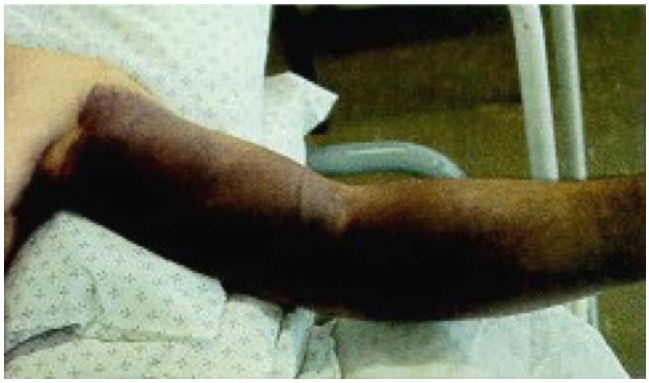

Fig. 5. Massive cutaneous ecchymosis on the arm of a CCHF patient, 7–10 days after clinical onset. Photograph courtesy of Dr. Robert Swanepoel, National Institute of Virology, South Africa.

surprisingly, poor prognosis is associated with cerebral hemorrhage and massive liver necrosis in severe cases. Mortality rates for the various CCHF epidemics and outbreaks have varied greatly. The average mortality rate is often cited at 30–50% (Hoogstraal, 1979; Nichol, 2001); however, rates as high as 72.7% and 80% have been reported from the United Arab Emirates and China, respectively (Schwarz et al., 1997; Yu-Chen et al., 1985). Mortality rates of nosocomial infections are often much higher than those acquired naturally through tick bite. The exact reasons for this phenomenon are not known, but may simply relate to viral dose.

For those who do not succumb to the disease, the convalescence period begins about 15–20 days after onset of illness. It is generally characterized by prolonged and pronounced generalized weakness, weak pulse, and sometimes complete loss of hair. Additional sequelae can include polyneuritis, sweating, headache, dizziness, nausea, poor appetite, labored breathing, poor vision, loss of hearing, and loss of memory (Hoogstraal, 1979). These problems are rarely permanent, but may persist for a year or more.

7. Pathogenesis/clinical pathology

The pathogenesis of CCHF is poorly understood. Because CCHF occurs sporadically, and in areas where clinical pathology facilities are limited, complete autopsies are seldom performed on patients who die from the disease. Additional factors that hamper studies on CCHF include the need for specialized laboratories (i.e., biosafety level-4 (BSL-4) containment) and lack of available animal models of disease. Therefore, limited knowledge of pathogenesis is often obtained from blood changes and liver biopsies of CCHF patients. The most comprehensive study of the clinical pathology of CCHF was that of Swanepoel et al., in which observations were made on 50 CCHF patients from South Africa diagnosed from 1981 to 1987 (Swanepoel et al., 1989). Of the 50 patients studied, 15 died (30% mortality), although one of those patients acquired bacterial meningitis as a complication to surgery for cerebral hemorrhage. Factors contributing to a fatal outcome included cerebral hemorrhage, severe anemia, severe dehydration, and shock associated with prolonged diarrhea, myocardial infarction, lung edema, and pleural effusion. Patients who died developed terminal multiple organ failure, including cerebral, liver, and kidney failure and cardiac and pulmonary insufficiency (Swanepoel et al., 1989). Liver lesions vary from disseminated necrotic foci to massive necrosis. Necrotic hepatocytes appear as amorphous masses and there is little or no inflammatory response. In fact, in patients who died, there was also little evidence of an antibody response (Shepherd et al., 1988).

Capillary fragility is a common feature of CCHF, suggesting infection of the endothelium. This is surely where the alternative term “capillary toxicosis”, given to CCHF by the early Soviet workers, was derived (Chumakov et al., 1976). Endothelial damage would account for the characteristic rash (see Fig. 5) and contribute to hemostatic failure by stimulating platelet aggregation and degranulation, with consequent activation of the intrinsic coagulation cascade. Thrombocytopenia appears to be a consistent feature of CCHF infection (Swanepoel et al., 1987, 1989; Schwarz et al., 1997) and platelet counts can often be extremely low from an early stage of illness in fatal cases. Indeed, of the fatal CCHF cases in the South African study (Swanepoel et al., 1989), all had grossly abnormal indicators of coagulation system function from an early stage of illness. The major beneficial outcome of that study was the realization that disseminated intravascular coagulopathy (DIC) was noted as an early and prominent feature of the disease process in CCHF.

The characteristic endothelial damage seen in CCHF is not necessarily the result of direct infection of the endothelial cells by CCHFV. At least in the case of Ebola hemorrhagic fever, evidence is mounting that much of the cellular damage and resulting coagulopathy actually results from multiple host-induced mechanisms (Geisbert et al., 2003b). These include massive apoptosis of lymphocytes both intravascularly and in lymphoid organs (Geisbert et al., 2000); induction of proinflammatory cytokines, including tumor necrosis factor (TNF)-α (Hensley et al., 2002); and the dysregulation of the coagulation cascade leading to DIC. Recently, Geisbert et al. (2003a) identified a molecular trigger for DIC through the expression of tissue factor (TF) on the surface of Ebola virus-infected monocytes and macrophages. Interestingly, some authors are now recognizing the similarities between various viral hemorrhagic fevers (i.e., dengue and Ebola hemorrhagic fevers) and septic shock caused by severe bacterial infections (Bray and Mahanty, 2003; Geisbert et al., 2003a; Mahanty and Bray, 2004). Indeed, many of these same features are seen in CCHF, including DIC, vascular dysfunction, and shock. Perhaps, with future research, therapies specifically targeting some of these host-induced mechanisms will yield more effective treatments for CCHF.

8. Diagnosis

Early diagnosis is essential, both for the outcome of the patient and, because of the potential for nosocomial infections, to prevent further transmission of disease. Clinical symptoms and patient history, especially travel to endemic areas and history of tick bite or exposure to blood or tissues of livestock or human patients, are the first indicators of CCHF. The differential diagnosis should include rickettsiosis (tick-borne typhus and African tick bite fever), leptospirosis, and borreliosis (relapsing fever). Additionally, other infections, which present as hemorrhagic disease such as meningococcal infections, hantavirus hemorrhagic fever, malaria, yellow fever, dengue, Omsk hemorrhagic fever, and Kyasanur Forest disease should be considered. In Africa, Lassa fever and infection with the filoviruses, Ebola and Marburg, must also be included in the differential diagnosis.

8.1. Laboratory diagnosis

8.1.1. Virus isolation

Any attempts at isolating and culturing the virus should only be performed in a maximum biocontainment laboratory (i.e., BSL-4). The traditional method for CCHFV isolation has been by intracranial (i.c.) or intraperitoneal (i.p.) inoculation of a sample (e.g., blood from an acute-phase patient or ground tick pools) into newborn mice. Isolation in cell culture is far simpler and provides a more rapid result, but is generally considered less sensitive (Shepherd et al., 1986) and can generally only detect high concentrations of virus. Nevertheless, virus can be isolated from blood and organ suspensions in a wide variety of susceptible cell lines including LLC-MK2, Vero, BHK-21, and SW-13 cells with maximal virus yields (10^7–10^8 plaque-forming units/ml) after 4–7 days of incubation (Nichol, 2001). Depending on the cell line and strain, the virus may produce little or no cytopathic effect (CPE) and develop into a noncytopathic persistent infection of the cells; however, virus can be identified by performing immunofluorescence assay (IFA) with specific monoclonal antibodies. Additionally, CPE and the visualization of plaques may occur only after serial passage of virus (Shepherd et al., 1986).

8.1.2. Immunological assays

Serologic tests used to study and diagnose CCHFV infection before 1980, such as complement fixation, immunodiffusion, and hemagglutination inhibition, suffered from a lack of sensitivity and reproducibility (Hoogstraal, 1979). Similarly, the neutralizing antibody response is weak and difficult to demonstrate in CCHF infections. These problems were largely overcome with the introduction of the indirect IFA (Zgurskaya and Chumakov, 1977) and the development of enzyme-linked immunoassays for detecting IgG and IgM antibodies (Donets et al., 1982). Both IgG and IgM antibodies are detectable by IFA by about 7 days after onset of illness and are present in the sera of survivors by day 9 (Shepherd et al., 1989). The IgM antibody declines to undetectable levels by the fourth month after infection, and IgG titers may also begin to decline gradually at this time, but remain demonstrable for at least 5 years. Recent or current infection is confirmed by demonstrating seroconversion, or a fourfold or greater increase in antibody titer in paired serum samples, or IgM antibody in a single sample (Swanepoel, 1995). An antibody response is rarely detectable in fatal cases and diagnosis is usually confirmed by isolation of the virus from the serum or liver biopsy specimens. Recently, new immunological assays incorporating recombinant CCHFV nucleoprotein have been developed and used in an IFA (Saijo et al., 2002b) or in an ELISA (Saijo et al., 2002a; Tang et al., 2003) to detect serum antibodies from infected patients.

8.1.3. Molecular diagnostic assays

Molecular-based diagnostic assays, such as the reverse transcription-polymerase chain reaction (RT-PCR), provide a useful complement to serodiagnosis and now often serve as the front-line tool in the diagnosis of CCHF, as well as other viral hemorrhagic fevers (Drosten et al., 2003). The benefits of using such assays are many. Because RT-PCR detects the genetic material of the virus, and can be designed to be highly specific, it is possible to make a presumptive diagnosis of CCHF without the need to culture the virus, which would require the use of specialized biocontainment laboratory facilities. Indeed, due to the high sensitivity of RT-PCR, positive results can often be obtained from samples which are culture negative (Schwarz et al., 1996). In addition, the assay can be applied retrospectively to stored serum samples. In one such study, viral RNA could be detected in samples up to day 16 of illness; whereas, infective virus was progressively cleared from the serum after the first week of illness (Burt et al., 1998). Another benefit to molecular diagnostic assays is their rapidity compared to virus culture, often allowing a presumptive diagnosis to be reported within 8 h of receiving the first specimen (Burt et al., 1998).

Likewise, RT-PCR assays for CCHFV have greatly enhanced epidemiological studies, for example, being able to detect viral nucleic acid directly from field-collected ticks. An added benefit of these techniques is that they allow for molecular epidemiology to be performed. Amplified viral complementary DNA (cDNA) can be sequenced and subjected to phylogenetic analysis. Using this approach, the source of a CCHF outbreak in the United Arab Emirates was determined (Rodriguez et al., 1997) and phylogenetically distinct viral variants were identified (Schwarz et al., 1996).

A further improvement on the conventional RT-PCR assay has been the development of automated real-time assays. The real-time PCR assay has many advantages over conventional RT-PCR methods, including lower contamination rate, higher sensitivity and specificity, and they are rapid, providing results in minutes instead of hours. Several investigators have reported the use of real-time PCR assays for detecting some viral causes of hemorrhagic fevers (Drosten et al., 2002a), including Ebola (Gibb et al., 2001; Towner et al., 2004; Weidmann et al., 2004), Rift Valley fever (Garcia et al., 2001), and dengue (Laue et al., 1999; Callahan et al., 2001; Houng et al., 2000) viruses. Drosten et al. (2002a) developed a one-step real-time RT-PCR assay for detecting CCHFV using primers to the nucleoprotein gene; however, they used the DNA-intercalating dye, SybrGreen I, for detecting the PCR product because no conserved binding site for a 5′-nuclease probe could be found. This problem has been partially solved by Garrison et al. (2003), who developed a real-time RT-PCR assay using TaqMan-minor groove binding protein (MGB) probe, allowing for greater specificity with a shorter probe length.

9. Treatment

Treatment options for CCHF are limited. Early remedies included giving rutin (a bioflavonoid compound found in buckwheat), ascorbic acid, and calcium chloride for the

treatment of the hemorrhagic syndrome. It was also suggested that with extensive blood loss, transfusions and blood substitutes such as polyglutin, plasma, and hemodes were necessary and intravenous injections of gelatin and aminocaproic acid were also indicated. Much emphasis was also placed on preventing reinfection, including the necessity of removing blood crusts from the oral cavity, brushing the teeth regularly, and painting with Vaseline any sores on the lips or tongue. There was an early recognition of the possible benefits of treatments using serum prepared from the blood of recovered CCHF patients or gammaglobulin obtained from immunization of horses (Hoogstraal, 1979). In more recent times, immunotherapy was attempted via passive transfer of CCHF survivor convalescent plasma (Vasilenko et al., 1990). Although seven patients with severe CCHF who received immune plasma recovered, this was an uncontrolled experiment, and firm evidence of its value is lacking. There is currently no specific antiviral therapy for CCHF approved for use in humans by the FDA. However, the antiviral drug, ribavirin, has shown the most promise over the years. It has been shown to inhibit in vitro viral replication in Vero cells (Watts et al., 1989a,b) and reduce the mean-time-to-death in a suckling mouse model of CCHF (Tignor and Hanham, 1993). Additionally, several case reports have been published that suggest oral or intravenous ribavirin is effective for treating CCHFV infections (Fisher-Hoch et al., 1995; Papa et al., 2002a; Mardani et al., 2003; Tang et al., 2003). For example, in Pakistan, three nosocomial cases of CCHF were treated with oral ribavirin for 10 days, and they made a complete recovery (Fisher-Hoch et al., 1995). More recently, in a large cohort study in Iran, the efficacy of oral ribavirin was 80% among patients with confirmed CCHF (Mardani et al., 2003). But, to date, no randomized, controlled studies have been performed to rigorously confirm the efficacy of ribavirin for treating CCHF. With the recent interest in CCHFV as a potential agent of bioterrorism/biowarfare, there have been increased research efforts by several groups. In particular, rapid methods of screening potential antiviral compounds are being applied to CCHFV (Paragas et al., 2004), as well as increased knowledge of the basic biology of the virus and its disease is being gained, which may lead to improved therapies, such as possible inhibitors of the viral protein processing (Pullikotil et al., 2004). Another area of interest with promise is the identification of interferon-induced proteins that inhibit viral replication, in particular, the Mx family of proteins. Mx proteins are interferon-induced GTPases that belong to the dynamin superfamily of large GTPases, which possess antiviral activity against a wide range of RNA viruses, including bunyaviruses and orthomyxoviruses (Haller and Kochs, 2002). Recently, Andersson et al. (2004a,b) showed that human MxA protein inhibits replication of CCHFV. They demonstrated that MxA co-localizes with the NP of CCHFV in the perinuclear regions of infected cells and that this interaction prevents replication of viral RNA and thereby inhibits the production of new infectious viral particles (Andersson et al., 2004a,b). There is clearly a critical need to identify new effective treatments for this disease, and if the past few years are any example, as more basic research is conducted on this virus, novel approaches to its control will surely evolve.

10. Prevention and control

10.1. Risk factors

There are several groups of individuals who are considered to be at-risk of contracting CCHFV. Specifically, people from endemic areas who are susceptible to tick bite, particularly from *Hyalomma* spp. ticks. These would include individuals who work outdoors, particularly those who work with large domestic animals. Although CCHFV has been isolated from numerous species of ticks (see Section 5), those of the *Hyalomma* genus are considered the primary vector in CCHF enzootic and endemic areas. The distribution of CCHFV coincides precisely with the distribution of *Hyalomma* ticks (Hoogstraal, 1956); therefore, there appears to be little or no risk in areas outside the known distribution of these ticks. Exposures such as crushing infected ticks and butchering infected animals have also been a frequent source of CCHFV infection. Other groups who are at-risk include those caring for CCHF patients. In fact, the risk of nosocomial infection in health-care workers is well documented and can be extremely high, especially during the hemorrhagic period of disease (Van Eeden et al., 1985; Fisher-Hoch et al., 1995; Papa et al., 2002a). This is exemplified by a nosocomial incident that occurred in the Central Government Hospital in Pakistan in January 1976 (Burney et al., 1980; Hoogstraal, 1979). A shepherd was brought to the hospital with typical CCHF symptoms and died the same night. The shepherd's father, who cared for his sick son at home, was hospitalized and died 2 days later despite intensive care and repeated blood transfusions. A female physician, who admitted the shepherd, when he vomited blood onto her face and hands, showed signs of CCHFV infection and was hospitalized, but ultimately recovered. The boy had surgery on the day of his hospital admission. The surgeon, who cut his finger while operating, died of CCHF 2 weeks later. An assistant surgeon who pricked his finger during the operation also contracted CCHF and was hospitalized; he later recovered. A nursing attendant who assisted in the operation died of CCHF 3 days later. The anesthesiologist also became ill and experienced bleeding from the gums, but recovered. Five of seven other persons in the operating theatre during the procedure also became ill and were hospitalized; all recovered. Of the 12 hospital personnel attending the shepherd, 10 became ill with CCHF; two died and eight recovered after severe illness. Another nosocomial outbreak occurred at Tygerberg Hospital in South Africa. Thirty-three percent of medical workers who had contact with patients through accidental needle pricks developed CCHF and 8.7% contracted disease by other contacts with the patients' blood (van de Wal et al., 1985).

Laboratory workers handling viral material are also at high risk of contracting the disease as evidenced by several cases of laboratory-acquired CCHF in Africa (Simpson et al., 1967), and several cases in Russia in which aerosol and/or droplet-respiratory route of infection were suspected (Hoogstraal, 1979). For these reasons, in the U.S., the Centers for Disease Control and Prevention (CDC) has classified CCHFV as a biosafety level-4 pathogen (Richmond and McKinney, 1999).

10.2. Control measures

The best means of preventing disease is to avoid or minimize exposure to the virus. This can be accomplished in a number of ways. Persons in high-risk occupations (i.e., slaughterhouse workers, veterinarians, sheep herders, etc.) should take every precaution to avoid exposure to virus-infected ticks or virus-contaminated animal blood or other tissues. For example, wearing gloves and limiting exposure of naked skin to fresh blood and other tissues of animals are effective practical control measures. Likewise, medical personnel who care for suspected CCHF patients should practice standard barrier-nursing techniques. Tick control may not always be practical in many regions of the world where *Hyalomma* ticks are most prevalent. However, acaricide treatment of livestock in CCHFV endemic areas is effective in reducing the population of infected ticks. Applying commercially available insect repellents (i.e., diethyl toluamide [DEET]) to exposed skin and the use of clothing impregnated with permethrin can give some protection against tick bites. As with other tick-borne diseases, inspection of one's body and clothes for ticks, and their prompt removal can minimize the risk of infection.

A suckling mouse brain, formalin-inactivated vaccine has been used in Bulgaria and other parts of Eastern Europe and the former Soviet Union. In the Rostov region of the former Soviet Union, 1500 persons received the vaccine and showed a high frequency of detectable antibody by the N test (Tkachenko et al., 1971). Likewise, vaccine was given to several hundred human volunteers in Bulgaria, with resulting high antibody induction (Vasilenko, 1973). With the relatively small target population of persons at-risk for contracting CCHFV, the large-scale development and production of a CCHF vaccine by modern standards seems unlikely.

11. Potential bioterrorism concerns

The highly pathogenic nature of the CCHFV has led to the fear that it might be used as an agent of bioterrorism and/or biowarfare and has resulted in its inclusion as a CDC/NIAID Category C Priority Pathogen. CCHFV can be transmitted from person to person, has a high case-fatality rate, and may be transmissible by small-particle aerosol; but, its inability to replicate to high concentrations in cell culture is cited as a major impediment to its development as a mass casualty weapon (Borio et al., 2002), and thus precludes its classification as a Category A or B pathogen. The highly lethal nature of the virus has restricted research to BSL-4 laboratories and has consequently had limited research investigations.

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